1316 cm^{-1}; MS, *m/e* (relative intensity) 217 (M^+, 4), 175 (13), 174 (100), 162 (16), 159 (28), 56 (15), 43 (36). Anal. Calcd for $C_{13}H_{15}NO_2$: C, 71.86; H, 6.86; N, 6.45. Found: C, 72.10; H, 6.98; N, 6.49.

The ketone **32** was reduced in a similar way as that of **7a** to give **33** (85%).

6,7-Dihydro-5-hydroxy-1,6,6-trimethyl-3,4-benzazepin-2-(5*H*)-one (33): mp 188–189 °C (from ethanol); ^{1}H NMR ($CDCl_3$) δ 1.14 (s, 3 H, MeC*Me*), 2.10 (s, 3 H, *Me*CMe), 2.62 and 2.90 (ABq, J = 14 Hz, 2 H, NCH_2), 3.08 (s, 3 H, NMe), 3.57 (s, 1 H, *H*COH), 4.20 (s, 1 H, OH), 7.2–7.8 (m, 4 H, Ar H); IR (KBr) 3305 (OH), 1625 (amide), 1595, 1428, 1256, 1058 cm^{-1}; MS, *m/e* relative intensity) 219 (M^+, 100), 190 (61), 161 (56), 44 (100). Anal. Calcd for $C_{13}H_{17}NO_2$: C, 71.20; H, 7.82; N, 6.39. Found: C, 71.20; H, 7.82; N, 6.40.

Irradiation of 1a with 2g. Irradiation of 1.9 g (12 mmol) of **1a** and 5 g (71 mmol) of 1-pentene (**2g**) in 400 mL of methanol or acetonitrile was performed. The results were described in the text.

Registry No. **1a**, 550-44-7; **1b**, 85-41-6; **1c**, 5493-24-3; **2a**, 100-42-5; **2b**, 98-83-9; **2c**, 530-48-3; **2d**, 110-83-8; **2e**, 513-35-9; **2f**, 115-11-7; **2g**, 109-67-1; (±)-**3a**, 95362-93-9; (±)-**3b**, 95362-75-7; (±)-**3c**, 95362-76-8; (±)-**3d**, 95362-77-9; (±)-**4a**, 95362-78-0; (±)-**4b**, 95362-79-1; (±)-**4c**, 95362-80-4; (±)-**4d**, 95362-81-5; **5a**, 68085-76-7; **5b**, 95362-82-6; **5c**, 95362-83-7; **6a**, 68085-75-6; **6b**, 95362-84-8; **6c**, 95362-85-9; (±)-**7a**, 95362-86-0; (±)-**7b**, 95362-90-6; (±)-**7c**, 95362-91-7; (±)-**7d**, 95362-92-8; **8**, 67643-55-4; (±)-(*R**,*R**)-**9**, 95362-88-2; (±)-(*R**,*S**)-**9**, 95362-87-1; (±)-(*R**,*R**)-**10**, 95363-20-5; (±)-(*R**,*S**)-**10**, 95362-89-3; (±)-(*R**,*R**)-**11**, 95362-94-0; (±)-(*R**,*S**)-**11**, 95362-95-1; (±)-(*R**,*R**)-**12**, 95362-96-2; (±)-(*R**,*S**)-**12**, 95406-38-5; (±)-(*R**,*R**)-**13**, 95362-98-4; (±)-(*R**,*S**)-**13**, 95362-97-3; (±)-(*R**,*R**)-**14**, 95363-00-1; (±)-(*R**,*S**)-**14**, 95362-99-5; (±)-(*R**,*R**)-**15**, 95363-02-3; (±)-(*R**,*S**)-**15**, 95363-01-2; (±)-(*R**,*R**)-**16**, 95363-03-4; (±)-(*R**,*S**)-**16**, 95363-04-5; **17**, 95363-05-6; (±)-*cis*-**18**, 95363-06-7; (±)-*trans*-**18**, 95363-07-8; (±)-**19**, 95363-08-9; **20**, 41976-80-1; (±)-**21**, 95363-09-0; (±)-**22**, 95363-10-3; **23**, 95363-11-4; **24**, 92172-54-8; (±)-**25**, 95363-12-5; (±)-**26**, 95363-13-6; (±)-(*R**,*R**)-**28**, 95363-14-7; (±)-(*R**,*S**)-**28**, 95363-15-8; **29**, 70113-69-8; (±)-(*R**,*R**)-**30**, 95363-16-9; (±)-(*R**,*S**)-**30**, 95363-17-0; (±)-**31**, 95363-18-1; **32**, 67177-35-9; (±)-**33**, 95363-19-2; **34**, 64837-64-5; phenanthrene, 85-01-8.

Sterols in Marine Invertebrates. 49.[1] Isolation and Structure Elucidation of Eight New Polyhydroxylated Sterols from the Soft Coral *Sinularia dissecta*

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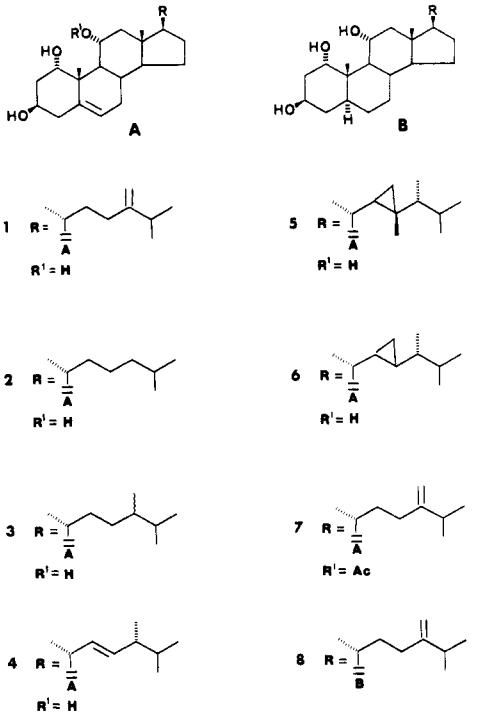
A new group of polyhydroxylated sterols—all of them possessing an 11α-hydroxy substituent of potential utility as corticosteroid intermediates—has been isolated from the soft coral *Sinularia dissecta*. Their general structure was deduced from spectral data (500-MHz and 360-MHz ^{1}H and ^{13}C NMR and MS) and their stereochemistry was determined by correlating the respective spectral data (^{1}H and ^{13}C NMR) with those of synthetic sterols with similar structure and known configuration.

Sterols with three or four hydroxyl functionalities have been previously reported in marine organisms such as soft corals,[2] sponges,[3] and starfish.[4] Our investigation of the sterol mixture from the Pacific soft coral *Sinularia dissecta* Tixier–Durivault collected near Palau led to the isolation and characterization of eight polyhydroxylated sterols (**1**–**8**), all with hydroxyl functions located in the nucleus including the important C-11 position. The crude sterol mixture contained, as major constituents, relatively polar metabolites. These polar fractions were separated into individual compounds by a combination of rapid-elution column chromatography (silica gel) and repeated reverse-phase high-performance liquid chromatography (HPLC) using several different solvent systems. The separation process was monitored at initial stages by TLC and in the latter stages by differential refractometry.

Extensive research has been performed in our laboratories to establish GC and HPLC standards to facilitate the structure determination of marine sterols. Unfortunately, in the present instance the typical standardized gas chromatography conditions were useless because of the excessively long retention times (up to 5 h) required at temperatures which do not decompose these sterols. The same remarks relate also to typical solvent systems for HPLC separations. In general, the best separations of



polyhydroxylated sterols were achieved by using acetonitrile–water systems and repeated separation with dif-

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ferent solvent combinations. Since the spectral data indicated that the major components of these polar fractions possessed virtually identical nuclei, but differed in the side chain, it was only necessary to settle the nuclear substitution pattern of the most abundant sterol **1**.

Structure Elucidation of 24-Methylenecholest-5-ene-1α,3β,11α-triol (1). The major component isolated from the polar fraction of the soft coral *Sinularia dissecta* was shown to be 24-methylenecholest-5-ene-1α,3β,11α-triol (**1**). The high-resolution mass spectrum displayed a molecular ion at m/z 430 corresponding to $C_{28}H_{46}O_3$. Successive losses of 18 mass units (m/z 412, 394, and 376) indicated the presence of three hydroxyl groups. The IR spectrum contained no carbonyl band but showed the existence of a methylene double bond (1635 and 885 cm^{-1}) and of hydroxyl groups (3400 cm^{-1}).

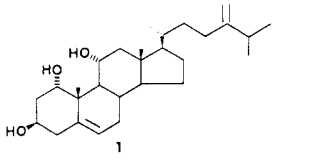
The 500-MHz ^1H NMR spectrum of **1** supported the existence of a terminal methylene group[5] (2 H, 4.643 and 4.713 ppm) and showed the presence of an additional trisubstituted double bond (1 H, bs at 5.565 ppm) characteristic of a Δ^5 vinylic proton,[6] as well as a terminal isopropyl group in the side chain (3 H, d, J = 6.82 at 1.024 ppm and 3 H, d, J = 6.82 at 1.021 ppm). Also present were a singlet at 0.678 ppm (18-CH_3) and a singlet at 1.144 ppm due to the C-19 methyl group. Three proton signals between 3.98 and 4.20 ppm were suggestive of the presence of secondary alcohols. A multiplet centered around 3.98 ppm had a complexity[7] normally seen for a 3α-carbinol proton, but the unusually high chemical shift suggested the presence of a perturbation in the vicinity of C-3. It was tempting to speculate that the additional secondary alcohol was located at positions 1, 2, or 4. However, double-resonance experiments proved that neither of the hydroxyls nor the olefinic proton were located next to each other. These experiments excluded all possible structures with 2,3-diol, 3,4-diol, Δ^5-3,7-diol, and Δ^4-3-ol groupings but suggested the presence of Δ^5 unsaturation and hydroxyls located at carbon atoms 1 and 3.[8]

The remaining hydroxyl group could only be located at C-11, C-12, C-15, or C-16. The small deshielding noted for the 1β-carbinol proton (observed chemical shift 4.21 ppm vs. expected values 3.84 ppm[8]) was best rationalized by placement of the remaining hydroxyl at the 11α-position. Moreover, the characteristic appearance[7] of the 11β-carbinol proton signal in the ^1H NMR spectrum strongly supported the location of the third hydroxyl group at C-11.

The ^{13}C NMR spectrum in $CDCl_3$ confirmed the presence of two carbon–carbon double bonds (δ 138.70, C-5; 124.50, C-6; 156.59, C-24; and 106.02, C-28). Assignment of the side chain carbons C-20 → C-28 in the ^{13}C NMR spectrum was based upon analogy to the known values for 24-methylenecholestane-1β,3β,5α,6β-tetrol.[9] To the best of our knowledge the presence or absence of the C-24 methylene group does not affect the chemical shifts of any carbon in the nucleus. Taking cholesterol as a starting structure,[10] the ^{13}C NMR spectrum could be completely assigned as shown in the Experimental Section.

Even stronger evidence about the hydroxyl group's position and configuration was based on examination of the NMR signals of the C-18 and C-19 protons. The chemical shift of the C-18 methyl group in **1** (0.678 ppm) was far too low for the presence of an 11β-hydroxyl group. Also the singlet assigned to the C-19 methyl protons (1.144 ppm) appeared at too high a field for an 11β-hydroxyl substituent. The chemical shifts of both angular methyl group protons in **1** were, however, in good agreement (Table II) with the respective calculated values using Arnold's substituent increment parameters[11] for Δ^5-1α,3β,11α-trihydroxycholestene. Finally, the complexity of the multiplets of the carbinol protons were exactly of the type exhibited by authentic cholest-5-ene-1α,3β-diol,[12] 5α-androstane-3β,11α-diol,[13] and 3β,11α,15α-trihydroxycholest-5-en-7-one.[14]

An independent confirmation of the structure assignment of rings A and B was provided by some simple chemical transformations of sterol **1**. Careful Jones oxidation of **1** afforded[15] the trione **9** with UV absorption at 242 mn (ε 12 500), suggesting the presence of a cross-conjugated[16] dienone system in the A ring. Its NMR assignment was in excellent agreement with the observed data of models 1,4-diene-3,11-dione[17] and 1,4-diene-3,6-dione.[18] All three signals of 1-H (δ 7.800, d, J = 10.30 Hz), 2-H (δ 6.288, dd, J = 10.30 and 2.00 Hz), and 4-H (δ 6.400, d, J = 2.00 Hz) in **9** were very close to the data of the related compounds:[17,18] δ 7.7, d, J = 10 Hz, 6.32, dd, J = 10.5 and 2 Hz, and 6.39, d, J = 2 Hz, respectively. Irradiation of 1-H (δ 7.800) resulted in decoupling of the signal of the neighboring C-2 proton which collapsed to a triplet δ 6.300 (J = 2.30 Hz).



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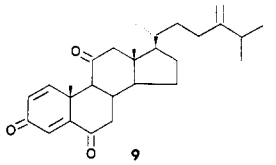

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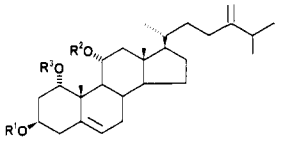
A separate series of experiments was based on selective acetylation of triol **1**. Under controlled conditions (dilute



1a R¹,R² = Ac, R³ = H
1b R¹,R²,R³ = Ac

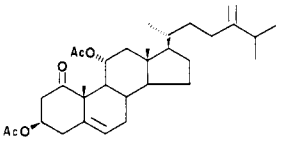


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Table I. Selected 360-MHz ^{1}H NMR Chemical Shifts ($CDCl_3$) of 1α,3β,11α-Triols[a]

sterol	C-1βH	C-3αH	C-11βH	C-6H	C-18H	C-19H	C-21H	C-26,27H	C-28H	other signals
1[b]	4.209 t (2.95)	3.981 m	4.083 m (10.25, 5.10)	5.565 ($w_{1/2}$ 12)	0.678 s	1.144 s	0.971 d (6.60)	1.024 d (6.82)	4.717 s	
								1.021 d (6.82)	4.651 s	
2	4.210 t (3.00)	3.980 m	4.080 m	5.565 ($w_{1/2}$ 12)	0.673 s	1.145 s	0.936 d (6.50)	0.867 d (6.61)		
								0.863 d (6.59)		
3	4.210 bs	3.980 m	4.080 m (10.00, 5.50)	5.564 ($w_{1/2}$ 12)	0.673 s	1.144 s	0.943 d (6.23)	0.856 d (6.79)	0.778 d (6.68)	
								0.786 d (6.60)		
4	4.207 bs	3.980 m	4.079 m	5.565 ($w_{1/2}$ 12)	0.685 s	1.144 s	1.030 d (6.64)	0.831 d (6.72)	0.907 d (6.83)	C-22,23H 5.178 m
								0.815 d (6.73)		
5	4.216 bs	3.980 m	4.080 m	5.570 ($w_{1/2}$ 12)	0.658 s	1.145 s	1.028 bs	0.953 d (6.05)	0.861 d (6.54)	C-23H 0.898 s
								0.937 d (6.31)		cyclopropyl: −0.117, 1 H, dd (6.00, 4.20); 0.220, 2 H, m; 0.476, 1 H, dd (9.00, 4.20)
6	4.210 bs	3.980 m	4.080 m	5.565 ($w_{1/2}$ 12)	0.636 s	1.140 s	0.942 d (6.60)	0.911 d (6.75)	0.857 d (6.81)	cyclopropyl: 0.135, 2 H, t (6.00); 0.300, 1 H, m; 0.540, 3 H, m
								0.890 d (6.80)		
7	3.699 t (3.00)	3.966 m	5.291 m	5.617 ($w_{1/2}$ 12)	0.774 s	1.118 s	0.928 d (6.43)	1.021 d (6.84)	4.713 s	
								1.016 d (6.82)	4.643 s	
8	4.050 bs		3.980, 2 H, m		0.650 s	0.895 s	0.951 d (6.48)	1.024 d (6.82)	4.720 s	
								1.019 d (6.81)	4.650 s	

[a] The chemical shift values are given in parts per million (ppm) and were referenced to $CDCl_3$ (7.260 ppm). The coupling constants are given in hertz and are enclosed in parentheses. [b] The NMR data reported for this compound were measured at 500 MHz.

Table II. Methyl Group Chemical Shifts of Trihydroxy Steroids Containing Different Configurations of 11-Position

sterol	C-18H, ppm	C-19H, ppm
1α,3β,11α-triol **1**	0.678	1.144
1α,11α-dihydroxycholesterol (calcd[11])	0.703	1.167
1α,11β-dihydroxycholesterol (calcd[11])	0.924	1.297

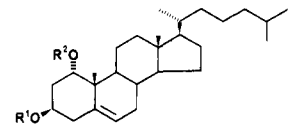
Table III. Comparison of the ^{1}H NMR Data ($CDCl_3$) for Hydroxy and Acetoxy Sterols 1, 1a, 1b, 14 (1α-Hydroxycholesterol[12]), 11, 12, and 13[a]

sterol	1β-H	3α-H	6-H
1	4.209 t	3.981 m	5.565
14	3.853 t	3.988 m	5.596
1a	3.720 t (3.00)	5.005 m	5.640
11	3.860 bs	5.033 m	5.609
1b	4.940 t (3.00)	4.827 m	5.632
12	5.058 t (3.00)	4.916 m	5.527
10		5.100 m	5.640
13		4.929 m	5.650

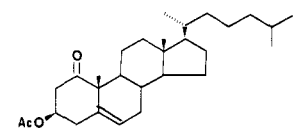
[a] The chemical shift values are given in parts per million (ppm) and were referenced to $CDCl_3$ (7.260 ppm). The coupling constants are given in hertz and are enclosed in parentheses.

ether–pyridine solution with 2 equiv of acetic anhydride), a readily separable mixture of the 3,11-diacetate **1a** and the 1,3,11-triacetate **1b** was obtained. Pyridinium chlorochromate oxidation of **1a** then afforded the diacetoxy ketone **10**.

Under identical conditions, similar transformations were performed with the known cholest-5-ene-1α,3β-diol[8] to afford the 3β-monoacetate **11** and the 1,3-diacetate **12**. In



11 R^1 = Ac, R^2 = H
12 R^1,R^2 = Ac



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comparing the chemical shifts of the 1β, 3α, 11β, and C-6 protons in pairs of respective compounds (i.e., **1** vs. cholest-5-ene-1α,3β-diol (**14**); **1a** vs. **11**; and **1b** vs. **12**), it was noted that the only significant difference was the chemical shift of 1β-protons (Table III). The shift toward the lower field in **1** (δ 4.209) and the high field shift in **1a** (δ 3.720) was caused by the deshielding effect of the oxygen function at C-11. The effect was the biggest in the 11α-hydroxy sterol **1**, while it was much smaller when the 11α-hydroxy group was acetylated (compounds **1a** and **1b**). Pyridinium chlorochromate oxidation of the 3β-monoacetate **11** again yielded keto acetate **13**. The correspondence of the NMR data of the model ketone **13** and diacetoxy ketone **10** also established their close structural relationship (Table III).

Table I contains the chemical shifts and observed multiplicity[7] ^{1}H NMR data of sterols **1–6**. These data show conclusively that they all had the Δ^5-1α,3β,11α-triol structure but different side chains. Their identification was simplified by reference to the ^{1}H NMR spectra and high-resolution MS data base of over 200 marine sterols isolated in our laboratories. In the 360-MHz spectra of triols **2**, **3**, and **4**, signals due to a terminal methylene group at 4.651 and 4.717 disappeared, while a doublet due to the secondary methyl group at C-24 was observed at δ 0.778 in **3**. In triol **4**, in addition to the above doublet at C-24 (δ 0.907), a multiplet appeared at δ 5.178 characteristic for the Δ^{22} double bond. Thus the structures of **3** and **4** were elucidated as 24(ξ)-methylcholest-5-ene-1α,3β,11α-triol and 24(*R*)-methylcholesta-5,22-diene-1α,3β,11α-triol, respectively. The side chain of compound **2** was of the unsub-

stituted cholesterol type with three doublets at δ 0.936, 0.867, 0.863 and two singlets due to the C-18 (δ 0.673) and C-19 (δ 1.145) methyl groups.

After compensating for the difference in unsaturation (Δ^5 vs. Δ^0), the ^{1}H NMR spectra of compounds **1** and **8** (Table I) were essentially identical. The lack of a Δ^5 double bond (in the nucleus of **8**) slightly affected the chemical shifts of the 1β- and 11β-protons (δ 4.050 and 3.980, respectively) while the C-19H signal appeared at a much higher field (at δ 0.895 in **8** vs. 1.144 in **1**). Thus, the structure of **8** was known to be 24-methylenecholestane-1α,3β,11α-triol.

As shown in Table I, the signals of both compounds **1** and **7** were closely related, except for the presence in **7** of a signal at δ 2.051 due to the acetoxyl methyl group. The high-field shift of the C-18H (δ 0.774) and C-19H (δ 1.118) signals in **7**, together with the low-field shift (δ 5.291) of the 11β-H signal, suggested that the hydroxyl group at the 11α-position in **1** was replaced by an acetoxy group in **7**. As could be expected, that change also influenced the chemical shift of the 1β-H, which displayed deshielding toward a higher field in relation to the respective chemical shift in the free alcohol **1**. These conclusions were confirmed by alkaline hydrolysis of **7** to **1**. Sterol **7**, therefore, is established as 24-methylenecholest-5-ene-1α,3β,11α-triol 11α-acetate.

The ^{1}H NMR spectra of sterols **5** and **6** displayed signals in the range of −0.117 to 0.540 pm confirming the presence of cyclopropane rings. The 360-MHz spectrum of compound **5** contained signals of cyclopropane protons: 1 H, dd at −0.117 ppm (J = 4.20 and 6.00 Hz), 2 H, m at 0.220 ppm, 1 H, dd at 0.476 ppm (J = 4.20 and 9.00 Hz); two singlets due to C-23H (0.898 ppm) and C-21H (1.028 ppm), and three doublets associated with terminal isopropyl group (0.937 ppm, J = 6.31 Hz and 0.953 ppm, J = 6.05 Hz) and the C-28 methyl substituted (0.861 ppm, J = 6.54 Hz). These data were in excellent agreement with those previously published for gorgosterol.[19] The side chain of compound **6** was identified as that of 23-demethylgorgosterol through the good agreement of its NMR signals (Table I) with those reported for natural 22(*R*),23(*R*),24(*R*)-demethylgorgosterol.[20]

The most interesting feature of the presently described *Sinularia* sterols is that they all possess the same 1α,3β,11α-trihydroxyandrost-5-ene nucleus and different side chains, which can be taken as *prima facie* evidence[19] that their organism possesses the enzyme system that converts dietary Δ^5-3β-hydroxy sterols into the same polyhydroxylated system. Of particular interest is the existence of the 11α-hydroxyl function, which offers a potential source for corticosteroid intermediates. The biological role of these polyhydroxylated sterols is not yet known.

Experimental Section

General Methods. Reverse-phase HPLC was performed by using Waters equipment (M6000 A pump, U6K injector, R401 refractometer), Whatman Partisil M9 10/50 ODS-2 column (9 mm i.d., 50 cm) and Altex Ultrasphere ODS (10 mm i.d., 30 cm) column. The eluent was 5–25% aqueous acetonitrile. The mass spectra were recorded at 70 eV on Varian MAT-44 or R-10-10B Ribermag (low-resolution) or Varian MAT-711 (high-resolution, double-focusing spectrometer equipped with a PDP-11/45 computer for data acquisition and reduction) mass spectrometers using a direct inlet system. The 360-MHz ^{1}H NMR spectra were recorded on a Brucker HXS-360 spectrometer, 500-MHz ^{1}H NMR spectra on a JEOL JNM-GX500 FT NMR spectrometer, and ^{13}C NMR spectra with a Nicolet NMC 300-MHz wide-bore spectrometer. The chemical shifts were given in ppm with $CDCl_3$ as internal standard, and the coupling constants were in hertz. IR spectra were run on a Nicolet MX-1 FT-IR instrument. UV spectra were obtained on a Hewlett-Packard 8450A UV/vis spectrophotometer using methanol as a solvent. Melting point were measured on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. Silica gel 60 (E. Merck, 70–230 mesh) was used for column chromatography.

Collection and Extraction of *Sinularia dissecta*. *Sinularia dissecta* Tixier-Durivault (1.6 kg wet) was collected at −12 m depth in September, 1979, in Palau, Western Caroline Islands. The sample was stored frozen, then freeze-dried, pulverized, and extracted 3× with $CHCl_3$. Removal of solvent under reduced pressure left a dark, viscous residue (22 g), which, by TLC analysis (silica gel, Et_2O), was found to contain relatively polar metabolites (the polyhydroxy sterols; R_f 0.1–0.2) as major constituents.

Chromatography. The extract (20 g) was initially chromatographed over TLC-grade (Merck) silica gel with isooctane/EtOAc mixtures using rapid-elution methods. Twelve fractions were obtained with the major polar compounds dispersed between fractions 8–12 (eluted with 80–100% EtOAc). Fractions 9–12 were combined to yield 2 g of a complex mixture.

Separation of the Polyhydroxylated Sterol Mixture. The polar fraction of the polyhydroxy sterols (homogeneous by TLC) was dissolved in acetonitrile and subjected to preparative reverse-phase HPLC on an ODS-2 column with acetonitrile/H_2O (9:1 as mobile phase). Further purification of the polyhydroxy sterols 1–8 was achieved by repeated HPLC with Altex ODS column with acetonitrile (**2**, **5**, **6**, and **8** sterols) and 5% aqueous acetonitrile (**1**, **3**, **4**, and **7** sterols) as the mobile phases.

24-Methylenecholest-5-ene-1α,3β,11α-triol (1): mp 161–162 °C; IR 3400, 1635, 885 cm^{-1}; for 500-MHz ^{1}H NMR, see Table I; 300-MHz ^{13}C NMR δ ($CDCl_3$) 74.45 (C-1), 38.11 (C-2), 65.98 (C-3), 41.93 (C-4), 138.70 (C-5), 124.50 (C-6), 32.41 (C-7), 31.76 (C-8), 48.21 (C-9), 42.74 (C-10), 67.82 (C-11), 50.57 (C-12), 42.93 (C-13), 55.66 (C-14), 24.24 (C-15), 28.34 (C-16), 55.49 (C-17), 12.50 (C-18), 19.25 (C-19), 35.60 (C-20), 18.68 (C-21), 34.48 (C-22), 30.91 (C-23), 156.59 (C-24), 33.74 (C-25), 21.81 (C-26), 21.96 (C-27), 106.02 (C-28); high-resolution EI/MS, m/z (assignment, relative intensity) 430.3448 (M^+, $C_{28}H_{46}O_3$, 3), 412.3352 ($M^+ - H_2O$, $C_{28}H_{44}O_2$, 100), 394.3246 ($M^+ - 2\,H_2O$, $C_{28}H_{42}O$, 74), 376.3147 ($M^+ - 3H_2O$, $C_{28}H_{40}$, 4), 340.3117 ($C_{25}H_{40}$, 10), 339.3054 ($C_{25}H_{39}$, 13), 285.1863 ($C_{19}H_{25}O_2$, 22), 267.1756 ($C_{19}H_{23}O$, 20), 233.1541 ($C_{15}H_{21}O_2$, 8), 227.1435 ($C_{16}H_{19}O$, 6), 209.1341 ($C_{16}H_{17}$, 8), 193.1223 ($C_{12}H_{17}O_2$, 7), 175.1118 ($C_{12}H_{15}O$, 14), 157.1024 ($C_{12}H_{13}$, 18).

Cholest-5-ene-1α,3β,11α-triol (2): for 360-MHz ^{1}H NMR, see Table I; high-resolution EI/MS, m/z (assignment, relative intensity) 418.3441 (M^+, $C_{27}H_{46}O_3$, 1), 400.3350 ($M^+ - H_2O$, $C_{27}H_{44}O_2$, 19), 382.3238 ($M^+ - 2H_2O$, $C_{27}H_{42}O$, 30), 364.3115 ($M^+ - 3H_2O$, $C_{27}H_{40}$, 6), 327.3045 ($C_{24}H_{39}$, 11), 233.1531 ($C_{15}H_{21}O_2$, 6), 227.1433 ($C_{16}H_{17}O$, 4), 209.1334 ($C_{16}H_{17}$, 7), 193.1220 ($C_{12}H_{17}O_2$, 7), 175.1120 ($C_{12}H_{15}O$, 14), 157.1006 ($C_{12}H_{13}$, 17), 107.0848 (C_8H_{11}, 35), 105.0696 (C_8H_9, 40), 95.0860 (C_7H_{11}, 58).

24(ξ)-Methylcholest-5-ene-1α,3β,11α-triol (3): for 360-MHz ^{1}H NMR, see Table I; high-resolution MS, m/z (assignment, relative intensity) 414.3512 ($M^+ - H_2O$, $C_{28}H_{46}O_2$, 2), 396.3376 ($M^+ - 2H_2O$, $C_{28}H_{44}O$, 11), 378.3283 ($M^+ - 3H_2O$, $C_{28}H_{42}$, 65), 376.3133 ($C_{28}H_{40}$, 41), 363.3057 ($C_{27}H_{39}$, 23), 279.2110 ($C_{21}H_{27}$, 26), 275.1797 ($C_{21}H_{23}$, 39), 209.1328 ($C_{16}H_{17}$, 67), 155.0858 ($C_{12}H_{11}$, 100), 81.0707 (C_6H_9, 62).

24(*R*)-Methylcholesta-5,22-diene-1α,3β,11α-triol (4): for 360-MHz ^{1}H NMR, see Table I; high-resolution MS, m/z (assignment, relative intensity) 430.3416 (M^+, $C_{28}H_{46}O_3$, 1), 412.3352 ($M^+ - H_2O$, $C_{28}H_{44}O_2$, 34), 394.3232 ($M^+ - 2H_2O$, $C_{28}H_{42}O$, 41), 376.3134 ($M^+ - 3H_2O$, $C_{28}H_{40}$, 40), 339.3046 ($C_{25}H_{39}$, 13), 285.1851 ($C_{19}H_{25}O$, 13), 269.1930 ($C_{19}H_{25}O$, 24), 251.1790 ($C_{19}H_{23}$, 56), 107.0862 (C_8H_{11}, 49), 81.0704 (C_6H_9, 100).

1α,11α-Dihydroxygorgosterol (5): mp 212–214 °C; for 360-MHz ^{1}H NMR, see Table I; high-resolution EI/MS, m/z (assignment, relative intensity) 458.3774 (M^+, $C_{30}H_{50}O_3$, 1), 440.3666 ($M^+ - H_2O$, $C_{30}H_{48}O_2$, 23), 422.3554 ($M^+ - 2H_2O$, $C_{30}H_{46}O$, 28), 324.2457 ($C_{23}H_{32}O$, 3), 310.2312 ($C_{22}H_{30}O$, 11) 269.1901 ($C_{19}H_{25}O$,

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10), 209.1329 ($C_{16}H_{17}$, 8), 175.1117 ($C_{12}H_{15}O$, 9), 159.1165 ($C_{12}H_{15}$, 12), 157.1011 ($C_{12}H_{13}$, 14), 145.1013 ($C_{11}H_{13}$, 23), 105.0703 (C_8H_9, 30), 97.1013 (C_7H_{13}, 37), 95.0858 (C_7H_{11}, 39).

1α,11α-Dihydroxy-23-demethylgorgosterol (6): for 360-MHz ^{1}H NMR, see Table I; low-resolution MS, m/z (relative intensity) 444 (M^+, 0.2), 426 (8), 412 (5), 394 (8), 383 (4), 373 (8), 255 (30), 55 (100).

24-Methylenecholest-5-ene-1α,3β,11α-triol 11α-acetate (7): for 360-MHz ^{1}H NMR, see Table I; high-resolution MS, m/z (assignment, relative intensity) 412.3348 ($M^+ - AcOH$, $C_{28}H_{44}O_2$, 22), 394.3217 ($M^+ - AcOH - H_2O$, $C_{28}H_{42}O$, 40), 392.3066 ($C_{28}H_{40}O$, 21), 388.2773 ($C_{28}H_{36}O$, 3), 376.3118 ($M^+ - AcOH - 2H_2O$, $C_{28}H_{40}$, 39), 374.2962 ($C_{28}H_{38}$, 21), 370.2643 ($C_{28}H_{34}$, 4), 361.2883 ($C_{27}H_{37}$, 10), 352.3144 ($C_{26}H_{40}$, 3), 339.3036 ($C_{25}H_{39}$, 16), 289.1832 ($C_{18}H_{25}O_3$, 5), 285.1838 ($C_{19}H_{25}O_2$, 36), 155.0859 ($C_{12}H_{11}$, 83), 121.1015 (C_9H_{13}, 57), 81.0702 (C_6H_9, 100).

24-Methylenecholestane-1α,3β,11α-triol (8): mp 185–186 °C; for 360-MHz ^{1}H NMR, see Table I; high-resolution EI/MS, m/z (assignment, relative intensity) 432.3585 (M^+, $C_{28}H_{48}O_3$, 2), 414.3517 ($M^+ - H_2O$, $C_{28}H_{46}O_2$, 15), 396.3388 ($M^+ - 2H_2O$, $C_{28}H_{44}O$, 7), 330.2562 ($C_{22}H_{34}O_2$, 23), 312.2469 ($C_{22}H_{32}O$, 13), 211.1493 ($C_{16}H_{19}$, 9), 175.1485 ($C_{13}H_{19}$, 14), 159.1168 ($C_{12}H_{15}$, 28), 145.1012 ($C_{11}H_{13}$, 29), 109.1013 (C_8H_{13}, 45), 105.0702 (C_8H_9, 48), 97.1013 (C_7H_{13}, 78), 95.0858 (C_7H_{11}, 87).

24-Methylenecholest-5-ene-1α,3β,11α-triol Triacetate (1b). The triol 1 (10 mg) was acetylated in pyridine (0.2 mL) by treatment with acetic anhydride (0.1 mL) at room temperature overnight. The pale yellow oil (12 mg) obtained after standard workup was separated by reverse-phase HPLC using 10% water–acetonitrile to give first a colorless amorphous solid (4 mg) which was identified as the diacetylated derivative **1a**: ^{1}H NMR δ 0.742 (s, 3 H, 18-CH_3), 0.927 (d, J = 6.42, 3 H, 21-CH_3), 1.015 (d, J = 6.82, 3 H, 27-CH_3), 1.019 (d, J = 6.78, 3 H, 26-CH_3), 1.123 (s, 3 H, 19-CH_3), 2.024 and 2.042 (2 s, 6 H, OAc), 3.720 (t, J = 3.00, 1 H, 1β-H), 4.643 and 4.712 (2 s, 2 H, 28-CH_2), 5.005 (m, 1 H, 3α-H), 5.283 (m, 1 H, 11β-H), 5.640 (m, 1 H, 6-H); low-resolution MS, m/z (relative intensity) 514 (M^+, 2), 454 ($M^+ - AcOH$, 12), 394 ($M^+ - 2AcOH$, 45), 376 ($M^+ - 2AcOH - H_2O$, 2), 267 (35), 175 (24), 135 (17), 43 (100). The next compound (8 mg) was identified as triacetate **1b**; ^{1}H NMR δ 0.759 (s, 3 H, 18-CH_3), 0.912 (d, J = 6.47, 3 H, 21-CH_3), 1.013 (d, J = 6.84, 3 H, 27-CH_3), 1.017 (d, J = 6.84, 3 H, 26-CH_3), 1.156 (s, 3 H, 19-CH_3), 2.011, 2.031, 2.067 (3 s, 9 H, OAc), 4.640 and 4.710 (2 s, 2 H, 28-CH_2), 4.827 (m, 1 H, 3α-H), 4.940 (t, J = 3.00, 1 H, 1β-H), 5.247 (m, 1 H, 11β-H), 5.632 (m, 1 H, 6-H); low-resolution MS, m/z (relative intensity) 556 (M^+, 1), 436 ($M^+ - 2AcOH$, 2), 376 ($M^+ - 3AcOH$, 56), 249 (27), 209 (33), 157 (46), 43 (100).

24-Methylenecholesta-1,4-diene-3,6,11-trione (9). A solution of triol 1 (5 mg) in 1 mL of acetone was treated with excess Jones reagent[21] and stirred at room temperature until the mixture turned orange-brown. The usual workup (ether for extraction) provided the colorless oil of triketone **9** (4 mg). Purification by reverse-phase HPLC using 10% aqueous acetonitrile afforded 2.5 mg of the triketone **9**: IR 1737, 1709, 1668 cm^{-1}; ^{1}H NMR δ 0.741 (s, 3 H, 18-CH_3), 1.022 (d, J = 6.82, 3 H, 27-CH_3), 1.027 (d, J = 6.83, 3 H, 26-CH_3), 1.395 (s, 3 H, 19-CH_3), 4.650 and 4.720 (2 s, 2 H, 28-CH_2), 6.288 (dd, J = 10.30 and 2.00, 1 H, 2-H; lit.[18] 6.32), 6.400 (d, J = 2.00, 1 H, 4-H; lit.[18] 6.39), 7.800 (d, J = 10.30, 1 H, 1-H; lit.[17] 7.7); UV λ_{max} 242 nm (ε 12 200); low-resolution MS, m/z (relative intensity) 422 (M^+, 6), 340 (3), 339 (18), 269 (15), 175 (24), 161 (21), 159 (21), 149 (9), 147 (27), 135 (100).

3β,11α-Dihydroxy-24-methylenecholest-5-en-1-one Diacetate (10). **1a** (6 mg) and 5 mg of pyridinium chlorochromate were dissolved in 0.5 mL of CH_2Cl_2 and the mixture was stirred at room temperature for 2 h. Ether was added and the residue was filtered off. After evaporation and purification by reverse-phase HPLC (acetonitrile), 5 mg (colorless amorphous solid) of **10** was obtained: IR 1740, 1727, 1237 cm^{-1}; ^{1}H NMR δ 0.765 (s, 3 H, 18-CH_3), 0.919 (d, J = 6.43, 3 H, 21-CH_3), 1.014 (d, J = 6.84, 3 H, 27-CH_3), 1.019 (d, J = 6.82, 3 H, 26-CH_3), 1.213 (s, 3 H, 19-CH_3), 1.877, 2.029 (2 s, 6 H, OAc), 4.643, 4.711 (2 s, 2 H, 28-CH_2), 5.095 (m, 2 H, 3α-H and 11β-H), 5.640 (m, 1 H, 6-H); low-resolution MS, m/z (relative intensity) 512 (M^+, 1), 428 (6), 393 (12), 392 (47), 308 (12), 266 (58), 265 (82), 209 (24), 171 (82), 69 (100).

Alkaline Hydrolysis of 7. A solution of **7** (2 mg) in EtOH (0.5 mL) was treated with 5% aqueous NaOH solution (0.2 mL) and the mixture was stirred at room temperature for 24 h. Water (1 mL) was added and the mixture was extracted twice with EtOAc (4 mL). Usual workup gave 1.5 mg of triol **1** as colorless needles (methanol). Spectral features obtained for the synthetic triol **1** were identical with those from the natural product.

Cholest-5-ene-1α,3β-diol 3β-Acetate (11). Acetylation of 1α-hydroxycholesterol (**14**)[12] (20 mg) with acetic anhydride (0.2 mL) in pyridine (0.4 mL) at room temperature overnight yielded 23 mg of mixture of acetate **11** and diacetate **12**. After purification by reverse-phase HPLC (methanol), two compounds were obtained: 7 mg of 3β-acetate **11**[22] [^{1}H NMR δ 0.673 (s, 3 H, 18-CH_3), 0.859 (d, J = 6.41, 6 H, 26- and 27-CH_3), 0.908 (d, J = 6.44, 3 H, 21-CH_3), 1.037 (s, 3 H, 19-CH_3), 2.028 (s, 3 H, OAc), 3.860 (bs, 1 H, 1β-H), 5.033 (m, 1 H, 3α-H), 5.609 (m, 1 H, 6-H)] and 15 mg of 1α,3β-diacetate **12**[22] [^{1}H NMR δ 0.660 (s, 3 H, 18-CH_3), 0.857 (d, J = 6.72, 3 H, 27-CH_3), 0.860 (d, J = 6.53, 3 H, 26-CH_3), 0.893 (d, J = 6.60, 3 H, 21-CH_3), 2.010 and 2.023 (2 s, 6 H, OAc), 4.916 (m, 1 H, 3α-H), 5.058 (t, J = 3.00, 1 H, 1β-H), 5.527 (m, 1 H, 6-H).

3β-Hydroxycholest-5-en-1-one Acetate (13). Sterol **11** (10 mg) and 8 mg of pyridinium chlorochromate were dissolved in 1.0 mL of CH_2Cl_2 and the mixture was stirred at room temperature for 2 h. Ether was added and the residue was filtered off. After evaporation and HPLC purification, 9 mg of **13**[22] was obtained: ^{1}H NMR δ 0.679 (s, 3 H, 18-CH_3), 0.860 (d, J = 6.52, 6 H, 26- and 27-CH_3), 0.904 (d, J = 6.45, 3 H, 21-CH_3, 1.264 (s, 3 H, 19-CH_3), 2.037 (s, 3 H, OAc), 4.929 (m, 1 H, 3α-H), 5.650 (m, 1 H, 6-H).

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Registry No. **1**, 95513-57-8; **1a**, 95513-58-9; **1b**, 95513-59-0; **2**, 95513-60-3; **3**, 95513-61-4; **4**, 95513-62-5; **5**, 95513-63-6; **6**, 95513-64-7; **7**, 95513-65-8; **8**, 95513-66-9; **9**, 95513-67-0; **10**, 95513-68-1; **11**, 35339-66-3; **12**, 35339-68-5; **13**, 35339-69-6; 1α-hydroxycholesterol, 26358-75-8.

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